Salmonella enterica in raw and boiled eggs: call for comprehensive preventive measures throughout the production and processing chain.

S.A. Mrope[1] and A.M.Lupindu[2]

[1]Department of Veterinary Microbiology, Parasitology & Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania

[2]Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine university of agriculture, Morogoro, Tanzania

***E-mail**: alupindu@suanet.ac.tz*

SUMMARY

Eggs are good source of proteins to human. However, consumption of contaminated eggs may lead to ill-health. *Salmonella enterica* typifies one of such pathogens causing food poisoning in humans. The present study aimed at assessing the bacteriological quality of eggs from chicken in respect to *Salmonella enterica*. A total of 570 chicken eggs were collected and examined from November 2015 to May 2016 in Morogoro municipality. *Salmonella* was isolated from egg content and eggshell samples by conventional culture and biochemical methods. *Salmonella enterica* were confirmed to genus level by PCR targeting *invA* gene. *Salmonella enterica* were tested for resistance against common antimicrobial agents. *Salmonella enterica* was recovered from 11 out 30 sample pools, of which 6 (20.0%) from egg contents and 5 (16.7%) from eggshell surfaces. Eggs from local chicken breeds were highly contaminated (20.0%) than eggs from exotic chicken breeds (16.7%) whereas raw eggs were highly contaminated (26.7%) than boiled eggs (10%). Clean eggs were highly contaminated (20.0%) than faecal socked (13.3%) and cracked eggs (3.4%). *Salmonella enterica* were susceptible to Ciprofloxacin, Chloramphenicol, Gentamicin, Tetracycline, Sulfamethaxole-Trimethoprim, Imepenem, Cefotaxime, Ceftriaxone and Caftazidime, and resistant to Kanamycin. Presence of pathogenic Salmonellae in different forms of chicken eggs indicates that eggs are unwholesome for human consumption and control measures should be implemented throughout egg production chain to prevent human and animal salmonellosis.

***Keywords**: Public health, food safety, Salmonella, egg, chicken, Morogoro*

INTRODUCTION

Eggs are easily available and affordable source of nutrients including high quality protein, carbohydrate, minerals and vitamins that provides nutritional balance to elderly, growing children and low-income families (Applegate, 2000; Zaheer, 2015). Large global egg production of approximately 100 million tones (FAO, 2018) make it reliable food source. However, consumption of contaminated eggs is reported to cause human salmonellosis with salmonella enteritidis taking the upper hand among the host unrestricted serovars (Martelli & Davies, 2012). The reasons for high egg associated salmonella enteritidis infection includes its ability to persistently colonize the reproductive system (ovary and the oviduct) of laying hens including the parental breeder flocks (Thorns, 2000).

Consumption of contaminated eggs is a risk factors towards human infection of *Salmonella enteritidis*. This occurs when people consume eggs contaminated with *Salmonella enterica* as raw, undercooked,

cracked or fecal soaked (Edema & Atayese, 2006). Moreover, *Salmonella enterica* infection occurred when people eat raw eggs with a believes that it cures cough and softens singer`s voice, and addition of raw eggs into child meal such as porridge also increase nutritional values. These factors are potentiated by poor hygienic practices in farms and catering services, improper storage temperatures and contaminated environmental factors (Kimura *et al*., 2004; Marcus *et al*., 2007; Middlegon et al., 2014).

Salmonella enterica serotype *enteritidis* infection can induce self-limiting gastroenteritis or an asymptomatic carrier state (Martelli & Davies, 2012) but infections can cause severe illness in elderly, infants and immunosuppressed individuals such as HIV/AIDS (CDC, 2010; Meremo et al., 2012). Usually signs start 12-72 hours after consumption of contaminated material and include fever, abdominal cramp and diarrhea. Due to these syndromes overlapping different diseases, salmonella enteritis cases are confirmed by laboratory procedures by using fecal or blood samples (Meremo *et al*., 2012).

Apart from causing disease syndromes, *Salmonella enterica* can develop resistance to antimicrobials such that treatment becomes ineffective. Unregulated access and misuse of antimicrobial agents to treat bacterial infections are associated with emergence of drug resistance (Katakweba *et al.*, 2012). The antimicrobial resistance has been reported to spread between chicken, humans and environment (Akond *et al*., 2012; Yhiler & Bassey, 2015). Additionally, the use of animal products after the animals have been treated with antibiotics without observing the withdraw periods may be a cause of antimicrobial resistance. Therefore, surveillance for disease causing pathogens may give more informative outcome if accompanied by antimicrobial susceptibility testing.

Control of egg and chicken borne *Salmonella enterica* infection in human should focus on prevention of pathogen-human contact throughout the production chain. This should include improvement of biosecurity measures at farms, assessing the microbiological quality of eggs, proper food handling, poultry vaccination and surveillance (Chai *et al*., 2012)Despite the science-based evidence about risk factors for egg related food-borne diseases (wide spread guidance) and strategies to prevent salmonella infection, continuous surveillance of *Salmonella enteritidis* (zoonotic serovars among *Salmonella enterica* subspecies) in eggs production chain is important so as to safe guide the consumer community.

This study, therefore, aimed at assessing microbiological quality of different forms of eggs in Morogoro municipality with respect to Salmonella enterica. It is the first surveillance study that examines eggs ready for human consumption and put forward informed advice on salmonella food-borne infection prevention strategies.

MATERIAL AND METHODS

Study design and sampling

This cross-sectional study was conducted in Morogoro municipality from November 2015 to May 2016. It involved chicken farms and food catering centers such as restaurants within Morogoro municipality from which eggs were randomly collected. Permission to conduct this study was granted by Sokoine University of Agriculture and consent was given by all participants in study area. Sample size was determined as previously described by Thrusfield (Thrusfield, 2007) whereby expected prevalence of Salmonella, confidence level and precision were set at 50%, 95% and 5% respectively. Correlation between egg sources was accounted for by

using intra-cluster correlation coefficient of 0.18 and design effect of 1.89 (Otte & Gumm, 1997). The resultant sample size of 720 eggs was adjusted to 570 eggs by considering the proportion of sample clusters in the population (Thrusfield, 1997). The egg samples composed of 120 raw eggs from indigenous chicken breeds, 360 raw and 90 boiled eggs from exotic chicken breeds. A total of 480 raw eggs were collected from 12 farms while 90 boiled eggs were collected from six food catering centers. They were collected aseptically and placed into new trays then transported to Microbiology laboratory for analysis.

Physical examination of chicken shell eggs

All individual eggs were physically examined to detect the presence cracks, feces and black or green discoloration on the shell surfaces. The eggs were categorized as clean (330), faecal soaked (120), and cracked eggs (120).

Sample processing

The 570 chicken eggs were pooled using previously described protocol (Andrews et al., 2014; PHE, 2014) into 30 composite sample pools. Out of these pools, 18 were from raw exotic, 6 raw indigenous and 6 from boiled exotic chicken eggs. Four hundred eighty raw eggs were composed of batches of randomly selected 40 eggs from a household/farm. These 480 eggs were divided into sample pools of 20 eggs each. The 90 boiled eggs were pooled into six pools each containing 15 eggs. From each egg sample pool, one shell surface and one egg content composite sample were prepared.

Shell surface samples were collected aseptically by using a swabbing technique (PHE, 2014). Swabs of each egg shell surface were pooled by dipping them into 10 ml sterile buffered peptone water in screw capped bottles. After swabbing, unbroken eggs were washed by normal saline and submerged into disinfectant (3 parts 70% ethanol to 1 part iodine solution) for 10 seconds and then air dried (Andrews et al., 2014). Sterile eggs were broken aseptically and the content was homogenized for 2 minutes and dispensed into sterile 1000 ml capacity Schott bottles. The egg content pools were incubated at room temperature for 96 hrs in order to recover injured or non-viable cells due to egg processing.

Isolation salmonella from egg shell surface

Pools of shell surface swabs in 10 ml Buffered Peptone Water were incubated at 37°C for 24hrs for enrichment. The enriched samples were transferred in two selective enrichment broths; Selenite Broth (at 1:10 dilution) and incubated at 37°C for 24 hours and Rappaport Vasiliadis Soy Broth (at 1:100 dilution) and incubated at 42°C for 24 hours.
Cultures of selective enrichment broths (Selenite Broth and Rappaport Vasilliadis Soy broth) were inoculated onto Blood agar, MacConkey agar, Xylose Lysine Dextrose agar, Bismuth Sulphite agar and Xylose Lysine Tergitol (XLT4) and incubated at 37°C for 24hrs. Negative growth culture results were re- incubated for another 24hrs and results recorded.

Isolation salmonella from egg content

After room temperature incubation, raw egg content sample pools were mixed well by swirling and 25 ml of mixture were then transferred into 500 ml Schott bottle containing 225ml Tripticase Soy Broth (TSB) with FeSO4 (35mg), while for boiled eggs 25g of sample was added to TBS without FeSO4 and then incubated at 35°C for 24hrs (Andrews et al., 2014).

After incubation, the enrichment broth cultures of egg contents were sub cultured in Selenite Broth by adding 1ml of cultures in 9ml Selenite Broth and incubated at 37ºC. the same enrichment broth cultures were also subcultured in Rappaport Vasilliadis Soy broth by adding 0.1ml of the culture in 1ml of Rappaport Vasilliadis Soy broth and inoculated at 42°C for 24hrs.

The broth cultures were then inoculated on blood agar, MacConkey agar, Xylose Lysine Dextrose agar, Bismuth Sulphite agar and Xylose Lysine Tergitol (XLT4) and incubated at 37°C for 24hrs. The Negative cultures were re-incubated for another 24hrs.

Table 1: Macroscopic features of suspected *Salmonella* colonies on solid media

Media	Colony characteristics
MacConkey agar	NLF colonies (pale pink colonies)
Blood agar	Greyish, small, raised moist non-haemolytic colonies
Xylose Lysine Dextrose agar	Large glossy, pink colonies with or without black centre or completely black colonies
XLT4	Black colonies or black cantered with a yellow periphery, yellow to red
Bismuth Sulphite agar	black or green-grey colonies with metallic sheen

Macroscopic and microscopic identification of suspected Salmonella isolates

Suspected Salmonella isolates were picked from the cultures based on characteristic appearance on different routine and selective media as shown in table 1. *Salmonella typhimurium* (Surrey University, USA) and *Escherichia coli* (Sokoine University, Tanzania) were used as positive and negative controls respectively.Smears of the suspected colonies Gram stained and observed under the microscope. Salmonella suspected colonies were expected to be Gram negative (reddish-pink in color).

Biochemical identification of suspected Salmonella colonies

Suspected *Salmonella* isolates were characterized by use of different biochemical tests, namely Triple sugar iron (TSI), Lysine decarboxylase (LIA), Urease, catalase, IMVIC (Indole, Methyl Red, Voges Proskauer and Citrate). Suspected *Salmonella* isolates were expected to show features presented in table 2.

Table 2: Characteristics of *Salmonella* isolates on different biochemical tests

Test	Characteristics of suspected *Salmonella* isolates
Triple sugar iron	yellow butt
Lysine decarboxylase (LIA)	purple butt
Production of H_2S from TSI and LIA	Blackening
Indole test	violet colour at surface
Voges-Proskauer test	no colour change
Methyl red test	diffuse red colour
Simon's Citrate	growth, blue purple
Urease test	no colour change (not hydrolysed)
Catalase test	formation of bubbles
Motility test	fuzzy (motile) and non- fuzzy growth (non- motile)

Molecular analysis of *Salmonella* isolates

DNA of *Salmonella* isolates was extracted by boiling colonies at 95ºC for 10 minutes. Centrifugation was done at 14 000 x g for 1 minute and the supernatant was used as DNA template. The DNA amplification of the *invA* gene was carried out using *Spec* primer pair (Macrogen Inc. Seoul, South Korea). The forward primer sequence of 5' GTG AAA TTA TCC CCA TCG GGC AA-3' and reverse primer sequence of 5' TCA TCG CAC CGT CAA AAC C-3' targeted

an amplicon of 284 bp. The PCR was run in a total volume of 20µl with the initial concentration of 0.5µM of primers. The PCR reaction mixture comprised of 2µl of DNA template, and 18µl of prepared Master mix made by adding 1 µl volumes of primers, 10 µl Dream Taq Green PCR Master Mix (2x), and 7 µl Nuclease Free Water in which the latter two reagents were obtained from Thermo Fisher Scientific Invitrogen Company, Westburg. The PCR mixture were run for DNA amplification in TAKARA Thermal Cycler Dice Gradient TP600 (Takara Bio, Tokyo, Japan) by using the amplification conditions of 35 cycles, initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 1minute, annealing temperature at 62°C for 30 seconds, extension at 72°C for 30 seconds, final extension at 72°C for7 minutes, and holding time at 4°C (Jamshindi, Kalidari, & Hedayati, 2010). The PCR amplification of products were analyzed by electrophoresis using 1.5% agar rose gels and visualized under UV trans-illuminator in gel Doc. EZ Imager machine (Bio Rad, California, USA).

Antimicrobial susceptibility testing

Agar disc diffusion method was used to obtain antimicrobial susceptibility profile of Salmonella isolates by using protocol previously described (Adesiji et al., 2014) with some modifications. One pure colony of salmonella isolates and standard *S. Typhimurium* (USA, Suresh University), used as positive, were emulsified in 200µl sterile normal saline. Turbidity of bacterial suspension was adjusted to 0.5 Standard McFarland solution. The suspension of isolates and the control were spread on Muller Hinton agar and allowed to dry for 30 minutes. Antibiotics impregnated discs (Oxoid, England) were applied on the agar surface and incubated at 37°C for 24 hours.

Isolates were tested with Sulfamethaxazole-Trimethoprim (25µg), Chloramphenicol (C 10µg), Tetracycline (TE 30µg), Gentamicin (Cn 10µg), Ciprofloxacin (CIP 5µg), Imipenem (IMI 10µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Ceftriaxone (CRO 30µg) and Kanamycin (K 30µg). Antibiogram profile were determined based on diameter of inhibition zones for each respective drug in millimeters and interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2008).

Data analysis

Data analysis was done by using GenStat software (v.14). Descriptive statistics such as proportions was computed to check the magnitude of salmonella enterica isolation from the samples. Chi-square test was used to test for significance difference of Salmonella enterica isolates between egg categories at 5% significance level.

RESULTS

Visual examination of egg shell surfaces

Out of 570 eggs examined, 1 (0.57%) egg had green discoloration indicating that the egg was contaminated. Visually, a total of 330 eggs were clean, while 120 had cracks and 120 eggs were fecal soaked. The latter two egg categories were all raw.

Bacteria isolation and characterization

Eleven (11) suspected *Salmonella* isolates were identified in the present study based on colonial morphology and biochemical test results. These isolates were confirmed *Salmonella enterica* by PCR as they carried a 284 bp *invA* gene (Supporting Figure 1). Six of the salmonella isolates (20%) were from egg content sample pools while five isolates (16.7%) were from eggshell surface pool samples. Two sample pools had salmonella isolates from both egg contents and egg shell surface swabs. The results show that eggs from local chicken breeds were more contaminated (20.0%) than eggs from exotic chicken breeds (16.7%).

Furthermore, raw eggs were more contaminated (26.7%) than boiled eggs (10%). This relationship was statistically significant (Table 3). Interestingly clean eggs were more contaminated (20.0%) than faecal socked eggs (13.3%).

Table 3: Distribution of *Salmonella enterica* among egg categories

Egg category	Frequency (%)	Chi-square	p-value
Eggs from local chicken breeds	6 (20%)	0.37	0.54
Eggs from exotic chicken breeds	5 (16.67%)		
Raw eggs	8 (26.67%)	9.28	0.00
Boiled eggs	3 (10%)		
Fecal soaked	4 (13.3%)	1.62	0.20
Clean eggs	6 (20%)		
Cracked	1 (3.3)		

Antimicrobial Susceptibility Profile of Isolated Salmonella enterica

Antimicrobial susceptibility tests showed that all *Salmonella enterica* isolates were resistant to kanamycin and susceptible to at least nine of the rest of the drugs. All 11 (100%) *Salmonella enterica* isolates exhibited sensitivity to Imepenem (IMI), Chloramphenicol (C), Ciprofloxacin (CIP), and Sulfamethaxole-Trimethoprim (SXT). Ten out of eleven (91%) *Salmonella enterica* were sensitive to Ceftazidime (CAZ), Gentamycin (Gn), Tetracycline (Te) whereas nine of Salmonella isolates (82%) were sensitive to Cefotaxime (CTX) and Ceftriaxone (CRO) (Table 4).

Table 4: Patterns of antimicrobial susceptibility profiles of *Salmonella enterica* isolates

Status	K30	Gn10	CIP5	CTX 30	C10	Te	CA Z	IMI	SXT	CR O
Susceptible	0	10	11	9	11	10	10	11	11	9
Intermediate	0	1	0	2	0	1	1	0	0	2
Resistant	11	0	0	0	0	0	0	0	0	0
Total isolates	11	11	11	11	11	11	11	11	11	11

DISCUSSION

The present study assessed bacteriological quality of local and commercial chicken eggs in respect to pathogenic Salmonella and found *Salmonella enterica* in egg shells surfaces (16.7%) and egg contents (20%). Low prevalence of *Salmonella enterica* contamination of eggs shell surfaces and egg contents have been reported in other places. For instance, the prevalence of 1.6% has been report from egg shell surface and none from egg contents in Mashhad, Iran (Jamshindi et al., 2010) while as in Coimbatore, South India, the prevalence 5.9% from egg shell surfaces and 1.8% from egg contents has been reported (Suresh *et a*l., 2006).

This higher prevalence of *Salmonella enterica* in the current study could be due to different protocols used among studies whereby in the present study the pooling of samples were employed based on methodology previously described (Andrews et al., 2014; PHE, 2014) and the use of different enrichments media which increased chances of *Salmonella* recovery from eggs.

Isolation of Salmonella from clean eggs has been reported in Ethiopia at a prevalence of 2.5% (Bayu, Asrade, Kebede, Sisay, & Bayu, 2013) and in cracked eggs in Iran at a prevalence of 1.6% (Jamshindi et al., 2010).

These reports differ from the current results in that the present study has found high prevalence of salmonella in clean eggs (20%) than in cracked eggs (3.3%) and fecal soaked (13.3%). Apart from differences in isolation protocols, differences in chicken management systems among chicken farms and between countries may primarly account for the existing contrasts. All in all, high prevalence of salmonella in clean eggs in study area attracts attention of and reminds egg consumers not to take for granted clean looking eggs as far as probability of human infection is concerned.

Pooling of egg sample has been adopted in investigations to assess of microbial quality eggs and risk of salmonella transmission (Andrews et al., 2014; PHE, 2014). On the other hand, samples of individual eggs have been processed for the same purposes (Suresh et al., 2006). In pooled sample protocol each pooled sample is regarded as one composite sample. Presence of *Salmonella* from multiple eggs constituting a pooled sample is not quantitatively appreciated.

Hence, this approach assesses the quality as either at least one egg in a sample pool was contaminated or not. When compared to protocols which assess individual eggs, pooling of egg samples may under estimate the degree of contamination. Therefore, the levels of salmonella contamination in the study area may be higher than reported.

This may pose a great risk of infection to human beings who come into contact with contaminated eggs. Antimicrobial susceptibility testing of isolated pathogens is an important surveillance procedure which not only informs about the status but also suggests appropriate therapeutic options and predicts antimicrobial resistance trends. Most of the isolates in the present study were sensitive to most of antibiotics tested. These results are similar to report from Katsina State, Nigeria, (Abdullahi et al., 2014) where *Salmonella enterica* isolates were reported to be susceptible to first line drugs such as Chloramphenicol, and Sulfamethaxole-Trimethoprim and the drug of choice Ciprofloxacin. as drugs of choice. Similar sensitivity results of antibiogarm testing have reported in Iraq (Al- Ledeni, Khudor, & Oufi, 2014) where all *Salmonella* isolates were sensitive to current used drugs; third generation Cephalosporins; (CAZ, CTX and CRO) together with Ciprofloxacin and Imipenem and hence justifying their continual use in treating Salmonellae infections.

However, antimicrobial sensitivity results of this study are in disagreement with report from Mangalore, India (Adesiji et al., 2014) in which tested Salmonellae were highly resistance to Chloramphenicol, Caftazidime, Ciprofloxacin, Tetracycline and Sulfamethaxole-Trimeyhoprim. The present study reports isolation of pathogenic salmonella from shell surface and contents of both raw and boiled eggs collected from chicken farms and food catering centers. This finding suggests that contamination of eggs occurs during production and/ or handling or preparation of egg meals. These isolates have shown variable degrees of resistance to common antimicrobials in the study area. Therefore, there should be a continued surveillance to inform producers, processors and consumers of eggs about the risk of infection and call for strategies to prevent contamination of eggs throughout the production chain

.

REFERENCES

Abdullahi, B., Abdulfatai, K., Wartu, J. R., Mzungu, I., Muhammad, H. I. D., & Abdulsalam, A. O. (2014). Antibiotic susceptibility patterns and characterization of clinical Salmonella Serotypes in Katsina State, Nigeria - Google Search. *Afr. J. Microbiol. Res*., *8*(9), 915–921.

Adesiji, Y. O., Deekshit, V. K., & Karunasagar, I. (2014). Antimicrobial-resistant genes associated with Salmonella spp. isolated from human, poultry, and seafood sources. *Food Sci. Nutr.*, *2*(4), 436–442.

Akond, M. A., Shirin, M., Alam, S., Hassan, S. M. R., Rahman, M. M., & Hoq, M. (2012). Frequency of drug resistant Salmonella spp. isolated from poultry samples in Bangladesh. *S. J. Microbiol*, *2*(1).

Al- Ledeni, A. A., Khudor, M. H., & Oufi, N. (2014). Isolation and Identification of Salmonella spp. from poultry farms by using different techniques and characterization of their antimicrobial susceptibilities - Google Search. *Basic J. Vet. Res.*, *13*(1), 246–259.

Andrews, W. H., Jacobson, A., & Hammack, T. (2014). Salmonella. In *Laboratory Methods - Bacteriological Analytical Manual - Salmonella*. U.S. Food and Drug Administration: Silver Spring, MD, USA.

Applegate, E. (2000). Introduction: Nutritional and Functional Roles of Eggs in the Diet. *J. Am. Coll. Nutr.*, *19* (5).

Bayu, Z., Asrade, B., Kebede, N., Sisay, Z., & Bayu, Y. (2013). Identification and characterization of Salmonella species in whole egg purchased from local markets in Addis Ababa, Ethiopia. *J. Vet. Med. Anim. Health.*, *5*(5), 133–137.

CDC. (2010). Multistate outbreak of human *Salmonella Enteritidis* infections associated with shell eggs (final update). Retrieved July 1, 2018, from https://www.cdc.gov/salmonella/2010/shell-eggs-12-2-10.html

Chai, S. J., White, P. L., Lathrop, S. L., Solghan, S. M., Medus, C., McGlinchey, B. M., … Mahon, B. E. (2012). *Salmonella enterica* Serotype *Enteritidis*: Increasing Incidence of Domestically Acquired Infections. *Clin. Infect. Dis.*, *54*(5), 488–497. https://doi.org/10.1093/cid/cis231

CLSI. (2008). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard—third edition. Wayne, PA: Clinical and Laboratory Standards Institute.

Edema, M., & Atayese, A. (2006). Bacteriological Quality of cracked eggs sold for consumption in Abeokuta, Nigeria. *Int. J. Poult. Sci.*, *5*(8), 772–775.

FAO. (2018). FAOSTAT: Production: Livestock Primary: Eggs Production. Retrieved June 29, 2018, from http://www.fao.org/faostat/en/#data/QL

Jamshindi, A., Kalidari, G. A., and Hedayati, M. (2010). Isolation and identification of *Salmonella enteritidis* and *Salmonella typhimurium* from the eggs of retail stores in Mashhad, Iran using conventional culture method and multiplex PCR assay. *J. Food Saf.*, *30*(3), 558–568.

Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E., and Muhairwa, A. P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livest. Res. Rural. Dev.*, *24*(170).

Kimura, A. C., Reddy, V., Marcus, R., Cieslak, P. R., Mohle-Boetani, J. C., Kassenborg, H. D., Swerdlow, D. L. (2004). Chicken Consumption Is a Newly Identified Risk Factor for Sporadic *Salmonella enterica* Serotype *Enteritidis* Infections in the United

States: A Case-Control Study in FoodNet Sites. *Clin. Infect. Dis.*, *38*(3), 244–252.

Marcus, R., Varma, J. K., Medus, C., Boothe, E. J., Anderson, B. J., Crume, T., Angulo, F. J. (2007). Re-assessment of risk factors for sporadic *Salmonella* serotype *Enteritidis* infections: a case-control study in five FoodNet Sites, 2002–2003. *Epidemiol. Infect.*, *135*(01), 84.

Martelli, F., and Davies, R. H. (2012). *Salmonella* serovars isolated from table eggs: An overview. *Food Res. Int.*, *45*(2), 745–754.

Meremo, A., Mshana, S. E., Kidenya, B. R., Kabangila, R., Peck, R., and Kataraihya, J. B. (2012). High prevalence of Non–typhoid Salmonella bacteraemia among febrile HIV adult patients admitted at a tertiary Hospital, North-Western Tanzania. *Int. Arch. Med.*, *5*(1), 28.

Middlegon, D., Savage, R., Tighe, M. K., Vrbova, L., Walton, R., Whitfield, Y., Cowcroft, N. S. (2014). Risk factors for sporadic domestically acquired *Salmonella* serovar *Enteritidis* infections: a case-control study in Ontario, Canada, 2011. *Epidemiol. Infect.*, *142*(07), 1411–1421.

Otte, M. J., & Gumm, I. D. (1997). Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Prev. Vet. Med.*, *3*(1), 147–150.

PHE. (2014). *Detection of Salmonella species*. London.

Suresh, T., Hatha, A. A. M., Sreenivasan, D., Sangeetha, N., and Lashmanaperumalsamy, P. (2006). Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. *Food Microbiol.*, *23*(3), 294–299.

Thorns, C. J. (2000). Bacterial food-borne zoonoses. *Rev. Sci. Tech. Off. Int. Epiz*, *19*(1), 226–239.

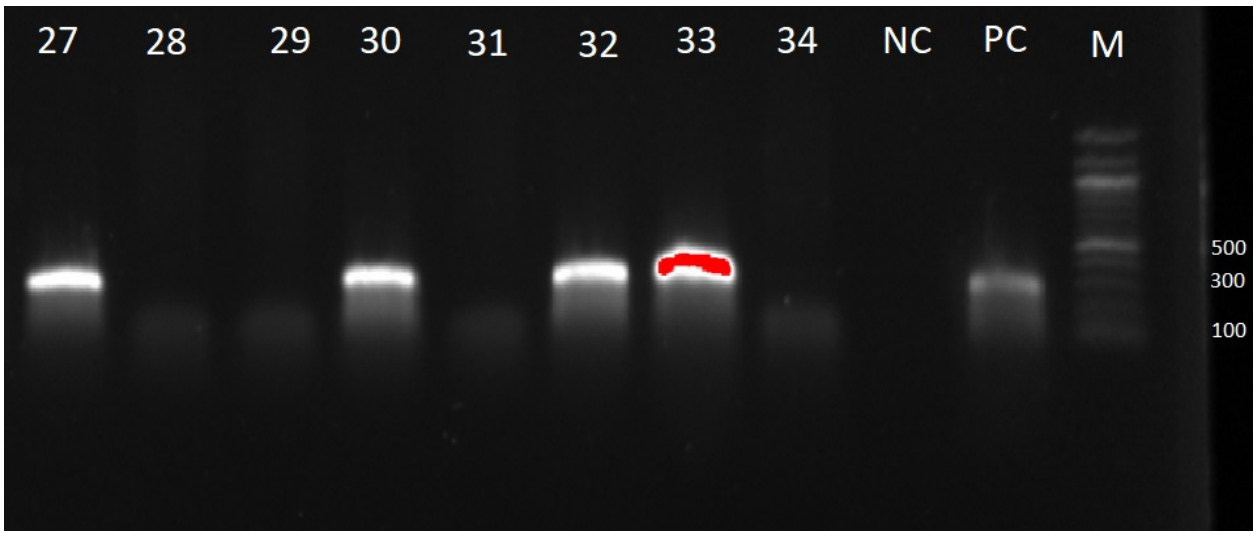
Thrusfield, M. V. (1997). *Veterinary epidemiology* (2nd edition). Wiley-Blackwell, Oxford.

Thrusfield, M. V. (2007). *Veterinary epidemiology* (3rd edition). Blackwell Science, Oxford.

Yhiler, N. Y., and Bassey, B. E. (2015). Antimicrobial Susceptibility Patterns of *Salmonella* Species from Sources in Poultry Production Settings in Calabar, Cross River State, Nigeria. *Am. J. Health Res.*, *3*(2), 76–81.

Zaheer, K. (2015). An Updated Review on Chicken Eggs: Production, Consumption, Management Aspects and Nutritional Benefits to Human Health. *Food Nutr. Sci.*, *6*(6), 1208–1220.

SUPPORTING INFORMATION



Supporting Figure 1: PCR gel picture for Salmonella enterica isolates: Lane 27, 30, 32 and 33 are positive isolates; lane 28, 29, 31 and 34 are negative isolates; lane NC = negative control; PC=positive control; M= I00 bp DNA ladder.